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High-Intensity HRP Chemiluminescence ELISA Substrate

Product Insert

www.Haemoscan.com

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Description

The HaemoScan High-Intensity HRP Chemiluminescence ELISA Substrate is an enhanced chemiluminescence formulation that produces high-intensity luminescence signals, providing high sensitivity in ELISA. The emitted light (peak emission at 425 nm) can be measured with a luminometer in black or white opaque microplates or in tubes. The substrate is specific for peroxidases such as horseradish peroxidase (HRP). Maximum signal intensity is reached shortly after initiation of the reaction and is stable over time (Figure 1). The substrate consists of two components, making it easy to use, and is stable at room temperature for at least six months.

Principle

Peroxidases such as horseradish peroxidase catalyse the oxidation of luminol to 3-aminophthalate by an oxidant such as perborate, which acts as a source of hydrogen peroxide. This reaction is accompanied by the emission of light at 425 nm, which can be measured with a luminometer. In contrast to colorimetric (chromogenic) substrates, which produce a coloured product that persists after the enzyme–substrate reaction has taken place, chemiluminescent substrates produce light during the enzyme–substrate reaction, which can be measured immediately.

Precautions and Handling

- The substrate is intended for research use only.
- Do not use the components beyond their expiry date.
- Do not combine reagents with different lot numbers.
- Dispose of the components in accordance with local and national regulations. For further information, please refer to the Safety Data Sheet (SDS), available at www.haemoscan.com.
- Store at room temperature, protected from direct sunlight and extreme temperatures. Keep containers tightly closed.
- Never pipette directly from the bottle; pour the required amount into a plastic reservoir. Do not return excess solution to the bottle. Wear disposable nitrile gloves and do not pipette by mouth.

Contents

- Perborate solution — 50, 125 or 250 mL per bottle
- Luminol solution — 50, 125 or 250 mL per bottle (amber bottle)

Example of a Microplate Test Procedure

1. Perform an ELISA in an opaque microplate, using an HRP conjugate for detection.
2. Mix equal volumes of Perborate solution and Luminol solution to obtain the substrate working solution.
3. Add 50–150 µL of working solution per well and mix on a plate shaker for 1 minute.

Note: Increase the volume of working solution as required for applications in test tubes.

4. Using a luminometer, measure the relative light units (RLU) within 5 minutes of adding the substrate to obtain the maximum signal intensity (Figure 1). For maximum sensitivity, measure the total light output; the peak emission wavelength (425 nm) is given for reference only.

Note: A purple/pink product may form during the reaction.

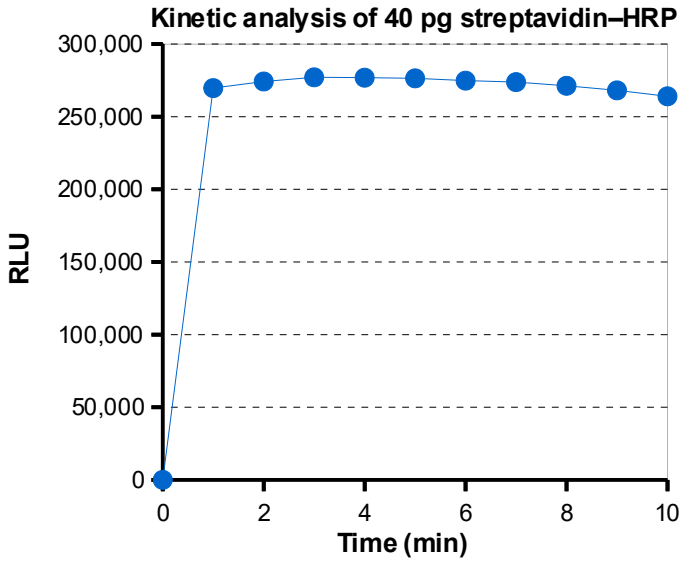

Figure 1. Rapid light generation with HaemoScan High-Intensity HRP Chemiluminescence ELISA Substrate. Streptavidin–HRP was diluted to 800 pg/mL in 0.1 M Tris, pH 8.5, and 50 µL (40 pg) was transferred to a white opaque Nunc MaxiSorp microplate. After addition of 50 µL of HaemoScan High-Intensity HRP Chemiluminescence ELISA Substrate, the microplate was incubated at room temperature on a microplate shaker for 10 seconds, and luminescence was measured at 1-minute intervals with a gain of 120 (GENios, Tecan Trading AG, Switzerland).